Comparing the effectiveness of processing parameters in pectin extraction from apple pomace

Hasan Ziari[1], Farzin Zokaee Ashtiani[1,2*], Mahdy Mohtashamy[1]

[1]Chemical Engineering Department, Amirkabir University of Technology, No. 424, Hafez Avenue, Tehran, Iran [2]Food Process Engineering and Biotechnology Research Centre, Amirkabir University of Technology, No. 424, Hafez Avenue, Tehran, Iran

Comparación de la eficacia de los parámetros de proceso en la extracción de pectina a partir de pulpa de manzana

Comparació de l'eficàcia dels paràmetres de procés en l'extracció de pectina a partir de polpa de poma

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RESUMEN

Se ha investigado el efecto de tres variables independientes en el proceso de extracción de pectina, incluyendo el tiempo de extracción (60 y 90 min), el pH de la disolución de extracción (1.5 y 2.0) y la temperatura del baño María (75 y 90 ºC), en el rendimiento y la calidad de la pectina de pulpa de manzana. El máximo rendimiento de pectina de 15.20%, se obtuvo a pH 1.5 extrayendo durante 90 min. a 90ºC, pero la máxima calidad de la pectina se obtuvo a pH 2.0, extrayendo durante 60 min. a 75ºC El análisis estadístico indicó que las variaciones de temperatura, pH y tiempo tienen el máximo efecto sobre el rendimiento y calidad de la pectina, respectivamente.

Palabras clave: pulpa de manzana, extracción de pectina, pH, calidad, temperatura, tiempo, rendimiento.

SUMMARY

The effects of three independent variables in pectin extraction process, including extraction time (60 & 90 min), pH of extraction solution (1.5 & 2.0) and water bath temperature (75 & 90ºC) on yield and quality of apple pomace pectin were investigated. The highest pectin yield of 15.20% was obtained at pH 1.5 for 90 min at 90°C, but the highest pectin quality factors were obtained at pH 2.0 for 60 min at 75°C. Statistical analysis indicated that variations of temperature, pH and time had the strongest effects on yield and quality of pectin, respectively.

Keywords: apple pomace. pectin extraction. pH. quality. temperature. time. yield.

RESUM

S'ha investigat l'efecte de tres variables independents en el procés d'extracció de pectina, incloent el temps d'extracció (60 i 90 min), el pH de la dissolució d'extracció (1.5 i 2.0) i la temperatura del bany María (75 i 90ºC), en el rendiment i la qualitat de la pectina de polpa de poma. El màxim rendiment de pectina de 15.20%, es va obtenir a Ph 1.5, extraient durant 90 min. a 90ºC, però la màxima qualitat de la pectina es va obtenir a pH 2,0, extraient durant 60 min. a 75ºC L'anàlisi estadística va indicar que les variacions de temperatura, pH i temps tenen l'efecte màxim sobre el rendiment i qualitat de la pectina, respectivament.

Paraules clau: polpa de poma, extracció de pectina, pH, qualitat, temperatura, temps, rendiment.

*Corresponding author: Tel: +98(21)64543124; Fax: +98(21)66405847; zokaee@aut.ac.ir

1. INTRODUCTION

Pectin is a group of complex colloidal polymeric carbohydrates, consisting largely of linear polymers of D-α-(1→4) anhydro-galacturonic acid. Pectin was first discovered by Vauquelin in 1790 who outlined its chemical nature, Braconnot isolated it in 1824 and gave it the name pectin (1). Pectin is part of a very complex structure in higher plants, which gives shape to the soft non-woody parts of the plant.

Due to gelating and/or stabilizing properties of pectins, they are widely used in food industry for a variety of purposes such as a gelling agent, thickener, texturiser, emulsifier and stabilizer (1,2), and have been used in various products such as jams and jellies, fruit juice, desserts and so on. In addition, non-food applications of pectins such as in pharmaceutical products have also been reported (3).

Pectins can be obtained on a laboratory scale from various sources by using different extraction methods such as hot acidic solution, cold diluted sodium hydroxide, cold and / or hot solutions of chelating agents (4). To produce pectin commercially, juicing operation waste products such as apple pomace or citrus peel are used as raw material and pectin is extracted in an acidic solution at elevated temperature. Operational extraction parameters such as pH, temperature and extraction time are the main factors that affect the yield and quality of pectin (5). Besides, pectin composition varies with the source from which it is extracted (6).

Wet apple pomace after juicing, is a source of pectin consisting of approximately 10–15% pectin on a dry weight basis (7), and is characterized in many applications by its superior gelling properties compared to citrus pectins (8).

Extraction of pectin from fresh peach pomace at different processing parameters such as temperature, acidity and extraction time was studied by Págan et al. (9). The effects of acid volume, acid-washing time and pH variation on the yield of dried mixed varieties of peach pomace during different stages of pectic substance extraction process, has been investigated by Faravash and Zokaee (10). Extraction of pectin from apple pomace at laboratory-scale was performed by Canteri-Schemin et al. (1) in order to observe the influence of citric acid concentration and reaction time on pectin yield. Garna et al. (11) investigated the effect of time, temperature and pH on the yield, degree of esterification, pectin content and natural sugar content of pectin extracted from apple pomace disregarding the washing steps of precipitated pectin using alcohol.

The main objective of the present research was to consider the effectiveness of time, pH and temperature on the yield, degree of methylation, pectin content and intrinsic viscosity of pectin extracted from apple pomace using statistical analysis . This study was accompanied by a concentration step and common alcohol washing steps, which weren't considered before. Following, is the detailed description of the extraction process.

2. MATERIALS AND METHODS

2.1. Materials

Hydrochloric acid, sulfuric acid and sodium hydroxide were obtained from Merck**.** 96% ethanol was obtained from a local supplier. Standard galacturonic acid sample was purchased from CP Kelco. All pH adjustments were done by a digital pH-meter (Metrohm 744) at room temperature.

2.2. Preparation of apple flours

Fresh apples were purchased from a local store and apple pomace was directly prepared after juicing. In order to neutralize pectolytic enzymes, the apple pomace was bleached in boiled water at 98ºC for 10 min (12) which altered the apple pomace color from brown to white. Then, the apple pomace was immediately placed in cold water until it cooled down to ambient temperature. Then bleaching was performed in 30ºC water with solid:liquid ratio 1:20 (w/v) at continuous stirring for 30 min (13). Consequently, soluble materials such as sugar were removed. The pomace was then drained and pressed to remove excess water. The next step was drying of pomace for 6 hours in ambient condition and then in oven at 50ºC, until constant weight of pomace was obtained. Then, dried pomace was ground via a hammer mill to pass a mesh size 60. The flour was kept in -20ºC in a non-air environment to be used later.

2.3. Pectin isolation

Pectin was extracted from apple flour (10g) using 0.1 N HCl as extracting agent (1:25 w/v) in a water bath apparatus equipped with a temperature controller system and constant stirring. The extraction was carried out at two different time periods (60 & 90 min), two different pHs (1.5 & 2.0) and two different temperatures (75 & 90ºC) (Table 1). After the extraction stage, the suspension were allowed to cool to room temperature in a water bath, and then centrifuged at 1500×*g* for 60 min. The supernatants were collected and then concentrated at 40ºC in a vacuum rotary evaporator (Büchi Co., Zurich, Switzerland) to one-fourth of its initial volume (14). Afterwards, pH of these dispersions were adjusted to 2.0 by a solution of 0.1 N NaOH and then pectin was precipitated by addition of 2x volume 96% ethanol to the dispersions. The resulting two-phase system was allowed to settle for 12 hours at room temperature. The precipitate was collected by filtration and a washing process was performed twice with 60% ethanol and twice with 70% ethanol, respectively, prior to a final washing with 96% ethanol. All washing steps were accompanied by stirring for 30 min to remove impurities such as monosaccharides and disaccharides (13). Thereafter, the pectin was pressed to remove excess alcohols and then placed in a vacuum oven (H.Jürgens, D2800 Bremen, Germany) at 40ºC until its weight became constant (1). Then the dried pectin was weighed with a digital scale, comminuted by a hammer mill and kept in -20ºC in a non-air environment for further analyses.

2.4. Determination of pectin yield and quality

2.4.1. Pectin yield

Pectin yield (w/w) was expressed as the ratio of dried pectin weight obtained after extraction to the initial apple flour weight at the start of the experiment.

2.4.2. Pectin contents

Pectin content of the sample, as a percentage of anhydrogalacturonic acid (AGA), was determined by a colorimetric method using m-hydroxydiphenyl (15).

***Table 1**. Effects of variation in time, pH and temperature on pectin yield and quality*

Extraction Conditions			Analysis Parameters				
pH	Temperature (°C)	Time (min)	%Yield (w/w)	% AGA[1]	% DM[2]	IV[3] (dl/gr)	M_w[4] (Da)
1.5	75	60	10.75±0.22	80.87±1.87	80.00±0.93	5.00±0.11	124301
1.5	75	90	11.10±0.17	77.00±1.00	79.15±0.79	4.35±0.10	102712
1.5	90	90	15.20±0.20	65.00±1.00	66.10±1.00	2.70±0.09	53444
1.5	90	60	14.75±0.25	67.30±1.08	68.50±1.00	3.12±0.04	65000
2.0	75	90	10.30±0.17	79.00±2.00	81.00±0.81	4.55±0.09	108895
2.0	75	60	10.10±0.10	81.00±1.50	82.21±0.71	5.25±0.10	132892
2.0	90	60	13.63±0.19	66.32±1.00	73.00±0.90	3.34±0.04	71522
2.0	90	90	14.30±0.30	65.70±0.75	69.40±0.73	2.89±0.07	58661

[1] Anhydro-galacturonic Acid content of pectin
[2] Degree of Methylation
[3] Intrinsic Viscosity
[4] Viscosity average molecular weight

2.4.3. Degree of Methylation
Degree of methylation (DM) expressed as the ratio of esterified galacturonic acid groups to the total galacturonic acid groups, was determined by a titration method (16).

2.4.4. Intrinsic Viscosity
The intrinsic viscosity (IV) of water-soluble polysaccharides extracted from the apple pomace was measured using an Ubbelohde capillary viscometer (Size 58) as carried out by Págan and Ibarz (17). All viscosity data was obtained at 25°C. IV values were calculated based on the following equations:

$$\eta_{sp} = \frac{(\eta - \eta_s)}{\eta_s} \quad (1)$$

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{C} \quad (2)$$

where η is the viscosity of sample solution, η_s is the viscosity of solvent and $[\eta]$ is the intrinsic viscosity.

2.4.5. Viscosity Average Molecular Weight
The viscosity average molecular weight (M_w) of pectin was calculated by applying the Mark-Houwink equation (18) as below:

$$[\eta] = 9.55 \times 10^{-4} M_w^{0.73} \quad (3)$$

where $[\eta]$ is the intrinsic viscosity (ml/g) and M_w is the average molecular weight of viscosity (Dalton).

2.5. Statistical analysis
All statistical analyses (student's *t*-test in significance level of $p<0.05$) were performed using Minitab software (version 14, Minitab Inc.). A factorial design of experiments was conducted and analysis of variance (ANOVA) was generated for each one of the responses, Moreover, figures for relative comparison between independent effects of extraction parameters on pectin yield and quality factors were plotted. Relationship between coded values (CV) and corresponding real values of independent parameters are as follows:

CV	pH	Temp. (°C)	Time (min)
+1	2.0	90	90
-1	1.5	75	60

3. RESULTS AND DISCUSSION

The mean values of three replicates for each of the experiments are presented in Table 1. Independent variables include extraction time, pH of extraction solution, and water bath temperature. Response parameters include pectin yield and corresponding quality factors such as AGA, DM, IV and M_W that determine the functionality of pectin

3.1. Yield of pectin
Based on the results represented in Table 1, pectin yield varied from the lowest value of 10.10% (pH 2.0 for 60 min at 75°C) to the highest value of 15.20% (pH 1.5 for 90 min at 90°C).
Independent effects of extraction time, pH and temperature on pectin yield are shown in Fig. 1. According to Fig. 1, an increase of time increased the pectin yield slightly (pH and temperature remaining constant). The same result was observed by Garna et al. (11) in extraction of apple pomace pectin, as well as by Mesbahi et al. (19) in pectin extraction from sugar-beet pulp.

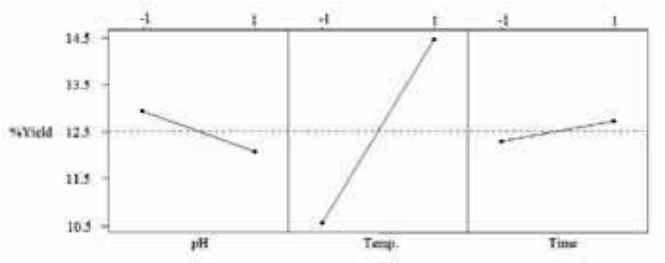

Figure 1. *Independent effect of time, pH and temperature on pectin yield.*

Since pectin extraction is a mass transfer process from solid phase to liquid phase, it could be terminated by time (leading to an equilibrium state). Thereafter, by extending the extraction time, the extracted pectins may degraded due to contact with hot acidic solution and they cannot be precipitated by adding alcohols (20). Hence, efficient extraction time should be taken into account as an important parameter in the extraction process. This phenomenon

has been noted by Phatak et al. (21) and De Vries et al. (22).

According to Fig. 1, the pectin yield increased with acid strength. According to Table 1, on average 12.95% and 12.08% (%w/w) of pectin were extracted, respectively, at pH 1.5 and pH 2.0. Similar results were observed by Garna et al. (11) in the extraction of apple pomace pectin, Mesbahi et al. (19) in pectin extraction from sugar-beet pulp as well as the study by Pagán and Ibarz (17) on pectin extraction from fresh peach pomace.

Using higher extraction pH value than the pH region of this study for hydrolyzing insoluble pectic substances is not applicable, because higher pH values or lower acidic power is equal to lower potential for hydrolyzing non-soluble pectic substance.

With regard to the influence of the extraction temperature, there was an increase in the pectin yield by increasing the temperature of extraction stage. Table 2, shows this significant effect. Mesbahi et al. (19), Garna et al. (11) and Pagán et al. (9) reported a similar effect of the temperature on pectin yield extracted from various sources.

Table 2. *ANOVA for pectin yield*

Source	DF[1]	SS[2]	MS[3]	F-value	*p*-value
pH	1	4.515	4.515	104.19	0.000
T	1	91.611	91.611	2113.9	0.000
time	1	1.046	1.046	24.13	0.000
pH×T	1	0.122	0.122	2.81	0.113
pH×time	1	0.002	0.002	0.04	0.839
T×time	1	0.122	0.122	2.81	0.113
pH×T×time	1	0.051	0.051	1.18	0.293

[1] *Degrees of freedom*
[2] *Sum of square*
[3] *Mean square*

3.2. AGA of pectin

The AGA content as the main component of pectin molecular chain varied from 65.00% (at pH 1.5 for 90 min at 90°C) to 81.00% (at pH 2.0 for 60 min at 75°C) (Table 1). Moreover, the effect of time, pH and temperature on the AGA of pectin is shown in Fig. 2.

Based on Table 1, increasing the temperature reduced the AGA content of extracted pectin. On average 66.08% and 79.47% AGA was obtained, respectively, at extraction temperature of 90°C and 75°C. This could be due to degradation of pectin's molecular chain to compounds with smaller molecular weight at higher temperatures, that would not precipitable by alcohols. Garna et al. (11) reported an increase in the AGA content of apple pomace pectin by increasing the temperature. El-Nawawi et al. (23) reported a bilateral effect of temperature on the AGA content of orange peel pectin. They observed that AGA content increased with the temperature increase from 50°C to 90°C. However, it dropped when temperature increased up to 110°C. Table 3 shows that temperature changes had the strongest effect on the variation of AGA (p<0.001).

Based on Table 1, on average 73.87% and 71.68% AGA was obtained, respectively, at extraction times of 60 and 90 min. Thus, an increase of time led to an insignificant decrease of AGA content of extracted pectin (Fig. 2). This result was in contrast to what Garna et al. (11) reported for apple pomace pectin extraction. Although their results showed a slight effect of time on the AGA content of apple pectin (p>0.05).

Variations of pH showed no significant change on the AGA content of pectin (p>0.05).

Overall, temperature, time and pH of extraction were the most effective parameters on the AGA variations of extracted pectin, respectively (Table 3).

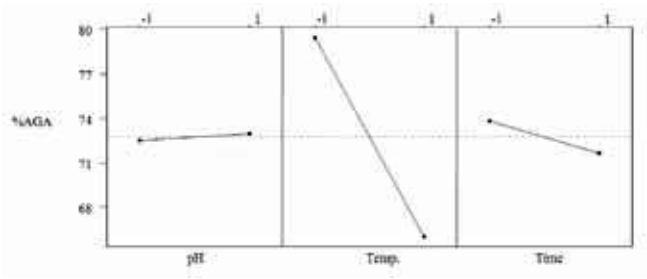

Figure 2. *Independent effect of time, pH and temperature on the anhydro-galacturonic acid content of pectin*

Since the pectin content in this work were all higher than 65%, they could be considered as commercial pectin products (24)

Table 3*. ANOVA for anhydro-galacturonic acid content of pectin*

Source	DF	SS	MS	F-value	*p*-value
pH	1	1.29	1.29	0.74	0.402
T	1	1075.22	1075.22	618.05	0.000
time	1	28.95	28.95	16.64	0.001
pH×T	1	2.18	2.18	1.26	0.279
pH×time	1	4.72	4.72	2.71	0.119
T×time	1	3.26	3.26	1.87	0.190
pH×T×time	1	0.01	0.01	0.01	0.932

3.3. DM of pectin

The DM of extracted pectin varied from 61.10% to 82.21% (Table 1). The highest DM was obtained at pH 2 for 60 min at 75°C. The lowest DM was obtained at pH 1.5 for 90 min at 90°C.

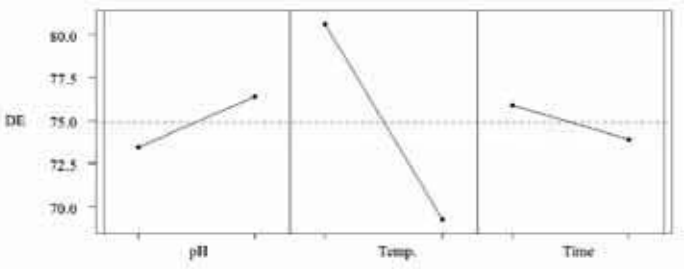

Figure 3. *Independent effect of time, pH and temperature on degree of methylation of pectin.*

Independent effects of time, pH and temperature on DM of extracted pectin are shown in Fig.3. As can be seen, (i) a temperature increase resulted in a significant decrease of DM. On average, 80.59% and 69.25% of DM were obtained, respectively, at 75°C and 90°C. (ii) An increase

of time was inversely proportional to DM but with much slighter effect than temperature. On average, 75.93% and 73.91% of DM were obtained, respectively, for 60 and 90 minutes of extraction time. (iii) An increase of pH led to an increase of DM, since it prevents the de-methylation of pectin. A same result was reported by Levigne et al. (25) on pectin extraction from fresh sugar beet as well as by Joye and luzio (24) on the lemon peel pectin extraction.
The effects of time, pH and temperature on DM of pectin in this study were qualitatively consistent with those reported by Garna et al. (11) on pectin extraction from apple pomace.
ANOVA for the DM of extracted pectin is presented in Table 4. The *F*-value of temperature shows its notably significant effect on the DM of pectin among other terms.
Since DM values in this study were higher than 50%, the apple pectins produced are high-methoxyl pectins.

***Table 4**. ANOVA for Degree of Methylation of pectin*

Source	DF	SS	MS	F-value	*p* -value
pH	1	52.75	52.75	70.41	0.000
T	1	771.57	771.57	1030.00	0.000
time	1	24.36	24.36	32.52	0.000
pH×T	1	5.25	5.25	7.00	0.018
pH×time	1	0.91	0.91	1.22	0.286
T×time	1	5.82	5.82	7.77	0.013
pH×T×time	1	0.26	0.26	0.35	0.561

3.4. IV and M_w of pectin

Variation of time, pH and temperature on the IV of extracted pectin is plotted in Fig. 4. According to Table 1, the highest IV of 5.25 dl/g was obtained in pH 2.0 for 60 min at 75°C, corresponding to the softest extraction conditions. On the other hand, the lowest IV value of 2.70 was obtained in the harshest extraction condition (pH 1.5 for 90 min at 90°C).

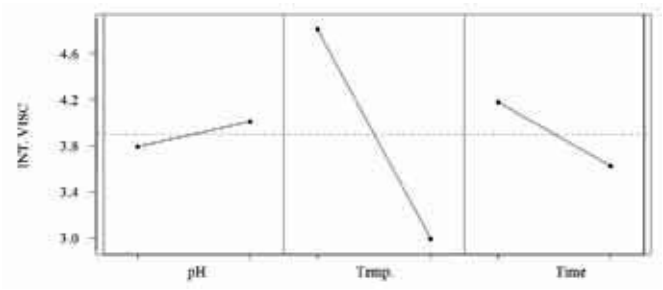

Figure 4. *Independent effect of time, pH and temperature on the intrinsic viscosity of pectin.*

Based on Table 1 and Fig. 4, (i) Increasing the temperature decreased the IV (and M_w) of pectin considerably owing to break down of pectin's molecular chains. On average, 4.79 and 3.01 (dl/gr) for IV of pectin was obtained, respectively, at 75°C and 90°C. (ii) Likewise, an increase of time decreased the IV of pectin but with slighter effect than temperature. (iii) Increasing the extraction pH increased the IV, but had the lowest independent effect.
Pagán et al. (9) showed the same influence of temperature and pH on the IV of pectin extracted from fresh peach pomace. In addition, the same effect of time, temperature and pH of extraction on the IV was reported by Emaga et al. (26) on pectin extraction from banana peels as well as by Levigne et al. (25) on pectin extraction from fresh sugar beet using both HCl and HNO_3 as extracting agents.
ANOVA for the IV of extracted pectin is presented in Table 5. Temperature variation had the strongest effect on the IV of pectin similar to DM and AGA among other terms.

***Table 5**. ANOVA for Intrinsic Viscosity of pectin*

Source	DF	SS	MS	F-value	*p* -value
pH	1	0.2993	0.2993	49.33	0.000
T	1	19.0817	19.0817	3145.33	0.000
time	1	1.9041	1.9041	313.86	0.000
pH×T	1	0.0020	0.0020	0.33	0.572
pH×time	1	0.0048	0.0048	0.79	0.386
T×time	1	0.0988	0.0988	16.29	0.001
pH×T×time	1	0.0011	0.0011	0.18	0.681

4. CONCLUSIONS

Operational parameters in pectin extraction process including time, pH and temperature have had different effects on the yield and quality of pectin qualitatively and quantitatively. Amongst them, temperature has shown the strongest effect in all analyses.
Overall, a temperature increase gave rise to increase of pectin yield, but AGA, DM and IV of pectin decreased as well. An increase of time had no significant effect on pectin yield, but decreased its AGA, DM and IV. Increasing the pH of extraction process resulted in a decrease in pectin yield but increased its AGA, DM and IV.
The highest pectin yield and also the lowest pectin quality factors (AGA, DM and IV) were obtained in the most drastic extraction conditions. It means that a higher pectin yield is not necessarily representative of pectin quality. Garna et al. (11) reported the same phenomenon and explained a co-precipitation of other components with pectin which resulted in a superficial increase in pectin yield.
It can be seen that various pectin yield and quality with desired end use could be achieved by altering the processing parameters of pectin extraction stage. Therefore, suitable operational conditions can be chosen based on actual need for pectin functionality.

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